

## **Anopheles embryo fixation for immunostaining V 2**

### **Background:**

The relatively impermeable chorion of *Anopheles gambiae* requires a different staining method than that used by *Drosophila melanogaster*. The following embryo fixation method is suitable for preparing *Anopheles gambiae* embryos for immunostaining and may be suitable for other anophelines or genera of mosquitoes. It was developed and used by Yury Goltsev [1] and further tested by John Yoder of the University of Wisconsin. As originally reported, embryos were collected for about 3 hours and then allowed to age to the desired developmental stage.

### **Solutions required:**

25% household bleach

Heptane

9% formaldehyde, adjust to pH=7 with NaOH.

Methanol

### **Fixation Procedure:**

- 1) Remove cup containing newly laid embryos from mosquito cage and age at room temperature.
- 2) Incubate eggs in 25% bleach for 75 seconds.
- 3) Rinse thoroughly with deionized water.
- 4) Place embryos in scintillation vials with 1:1 heptane: 9% formaldehyde, pH = 7. Shake on rotary platform for 25 minutes.
- 5) Remove embryos from mixture and replace with 1:1 heptane: deionized water.
- 6) Shake on platform an additional 30 minutes.
- 7) Remove water only (leaving heptane phase). Fill vial to the top with boiling deionized water. Incubate for 30 seconds.
- 8) Remove hot water only (leaving heptane phase) and replace with ice-cold deionized water.
- 9) Place vial on ice for 15 minutes.
- 10) Remove water and replace heptane.
- 11) Add an equal amount of methanol to create a 1:1 heptane: methanol mixture.
- 12) Let stand 10 – 15 minutes.
- 13) Remove embryos and wash several times with methanol.

At this point, the embryos can be stored at –20°C for several months.

The endochorion must be manually peeled away using fine needles and double stick tape before staining.

1. **Goltsev Y, Hsiong W, Lanzaro G, Levine M.: Different combinations of gap repressors for common stripes in *Anopheles* and *Drosophila* embryos.** Dev Biol. 2004 Nov 15;275(2):435-46.